Supplemental Data Inventory

We have attached supplemental figures and legends only. There are 5 main figures and 5 supplemental figures. We have moved these figures to supplemental for space constraints.

Supplemental figure 1: Shows the sequence of the fluorescent proteins used and the insertion sites for probes used and is associated with Figure 1 in the manuscript.

Supplemental figure 2: Shows the spectrum of new fluorescent protein described in this manuscript and is associated with Figure 2 in the manuscript.

Supplemental figure 3: Shows the method of fitting the response kinetics of the probes and the corresponding taus. This is associated to Figure 3 in the manuscript.

Supplemental figure 4: Shows a confocal image of a neuron labeled with an ArcLight probe and the lack of effect of ArcLight expression on action potential shape and size over time. It is associated with Figure 4 in the manuscript.

Supplemental figure 5: Shows the fluorescence response of an HEK293 cell expressing ArcLight A242 to the of application of an action potential-shaped voltage waveform. It is associated with Figure 5 in the manuscript.

Supplemental Figures

Figure S1. Protein sequences used in this study and structure of ArcLight probes A) Protein sequence alignment of ecliptic pHluorin and eGFP. Residue A227 is outlined with blue. Residues that are different between ecliptic pHluorin and eGFP are outlined with red. Residues in ecliptic pHluorin that were mutated to those seen in eGFP are outlined with thicker red. The residues that form beta sheets of the FPs are shaded with light green. **B)** Derivative probes of ArcLight S249 were generated by relocating the FP closer to the S4 domain of CiVS. The phosphatase domain of the CiVSP is deleted in all of our probes. The remaining voltage sensor domain (shaded in yellow) was conjugated with the super ecliptic pHluorin A227D (shaded with green) at different locations. The three amino acids, GDP, between the CiVS and the FP were translated from a BamHI restriction site, which was originally engineered for Mermaid, the parent probe of ArcLight.

Figure S2. Excitation and emission spectra and pH sensitivity of super ecliptic pHluorin and super ecliptic pHluorin A227D. A) Excitation and emission spectra of super ecliptic pHluorin (black) and super ecliptic pHluorin A227D (red**).** The emission wavelength for the excitation measurements was 550 nm. The excitation wavelength for the emission measurements was 465 nm. **B)** Emission spectra of super ecliptic pHluorin (left panel) and super ecliptic pHluorin A227D (right panel) in buffers with different pH: 9.5 (purple), 8.5 (blue), 7.5 (cyan), 6.5 (green), 5.5 (orange yellow), 4.5 (orange red) and 3.5 (red). **C)** The normalized fluorescence intensity *vs* pH for super ecliptic pHluorin (black) and super ecliptic pHluorin A227D (red).

Figure S3. Single or double exponential fits for the dynamics of ArcLight and fluorescence response dynamics of several probes used in this study. A) Bottom: Two presentations of a sample optical trace during depolarizing steps from -70 mV to 30 mV. The on time course can be fitted successfully with either a double (red) or a single exponential equation (black). Top: The residual analysis indicates that the double exponential fit is more accurate in the first 100 ms of depolarization. **B)** τ of the fast and slow components and the relative amplitudes of each component (mean ± SEM) from the double exponential fits for the following CiVS-based FP voltage sensors with : ecliptic pHluorin A227D inserted at S249 (n=8), ArcLight (n=9), ArcLight Q239 (n=6), ArcLight M240 (n=8), ArcLight K241 (n=7), ArcLight A242 (n=6), ArcLight S243 (n=7) The relative amplitude of the slow components are not given but can be determined by subtracting that of the fast component from one.

Figure S4. A neuron expressing the ArcLight Q239 probe visualized with confocal microscopy and lack of effect of ArcLight expression on action potential amplitude and duration. A) Inset: increased magnification of the boxed area illustrating membrane expression of ArcLight Q239 in a dendrite. Scale Bar = 10 μm. Lack of effect of transfection of ArcLight on action potential amplitude or width at half maximal amplitude **B)** Left: Amplitude of spontaneous action potentials (mean ± SEM) from patch clamp recordings of mouse hippocampal neurons *in vitro* in non-transfected (49 action potentials in 6 neurons), mock transfected (exposed to Lipofectamine 2000; 27 action potentials from 5 neurons) and ArcLight Q239 or A242 transfected neurons (91 action potentials in 16 neurons). Right: Action potential widths (mean ± SEM) at half maximum amplitude in mouse hippocampal neurons *in vitro*. **C)**

Shape of spontaneous action potentials in ArcLight Q239 transfected mouse hippocampal neurons are not significantly altered by 4 min of laser illumination. Time scale bar is 5 ms.

Figure S5. Fluorescence response of an ArcLight A242 expressing HEK 293 cell to depolarization with an action potential-like waveform. The upper trace is the fluorescence response of a single action potential-like voltage clamp waveform presented to a HEK 293 cell expressing ArcLight A242. The lower trace is the voltage waveform derived from a hippocampal neuron spontaneous action potential voltage recording. The fluorescence signal has had the bleach rate subtracted using a double exponential fit.

A

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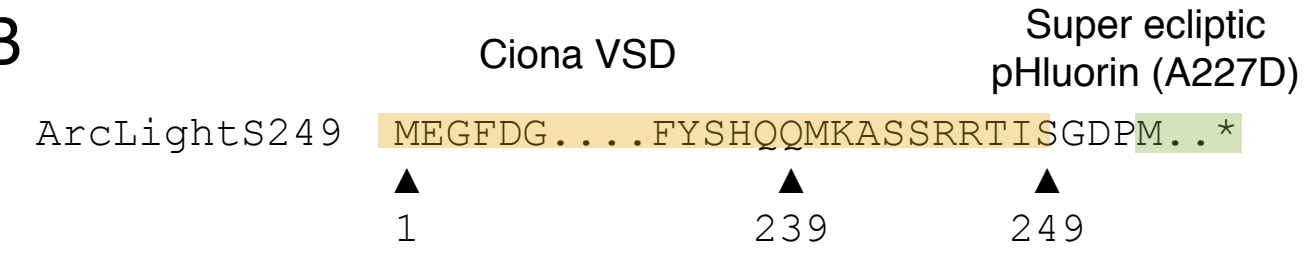
A227 | Mutations (ecliptic pHluorin to eGFP) described in this report

Additional differences between ecliptic pHluorin and eGFP | Beta sheets

B

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Arclight Q239  MEGFDG....FYSHQQGDPM..*
Arclight M240  MEGFDG....FYSHQQMGDPM..*
Arclight K241  MEGFDG....FYSHQQMKGDPM..*
Arclight A242  MEGFDG....FYSHQQMKAGDPM..*
Arclight S243  MEGFDG....FYSHQQMKASGDPM..*
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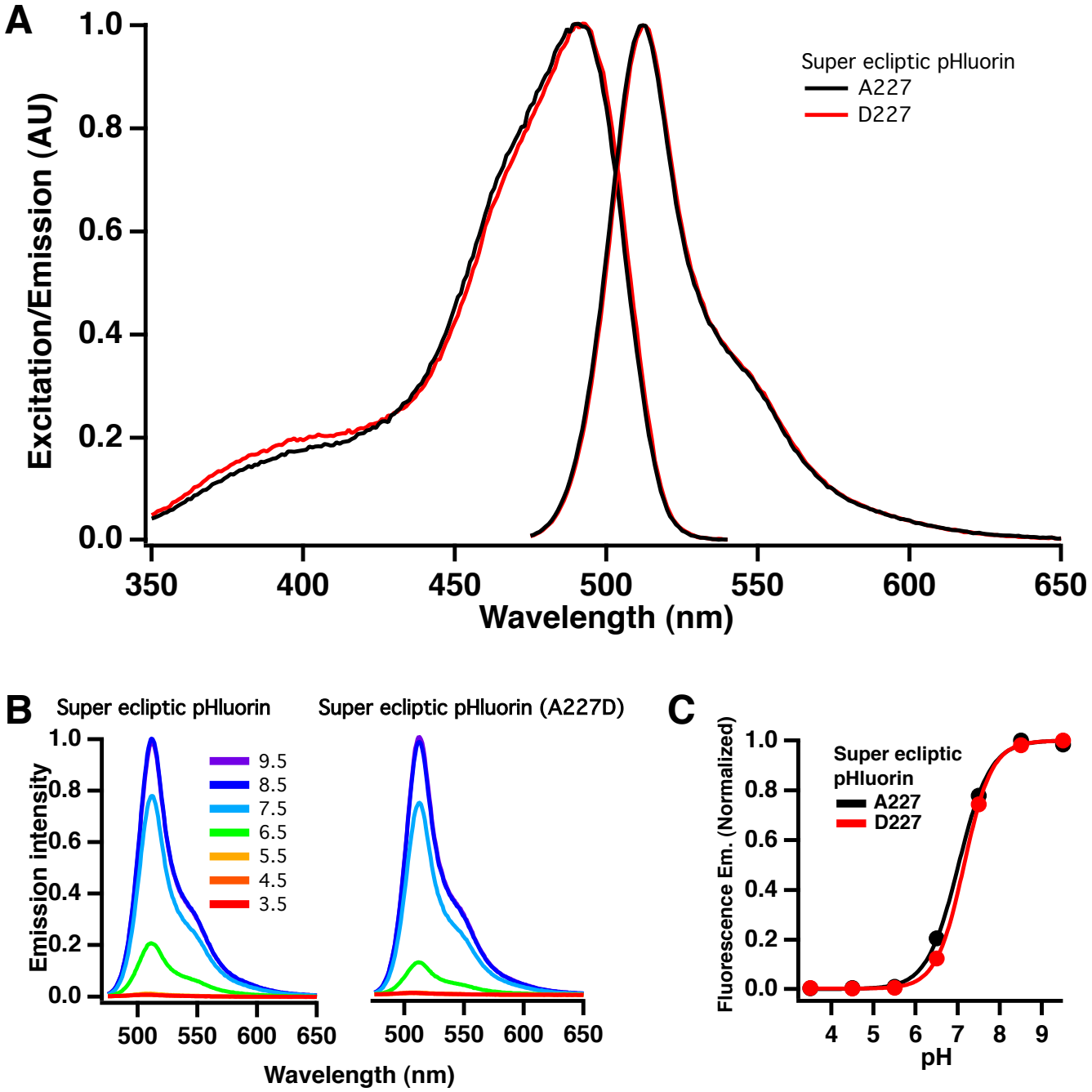
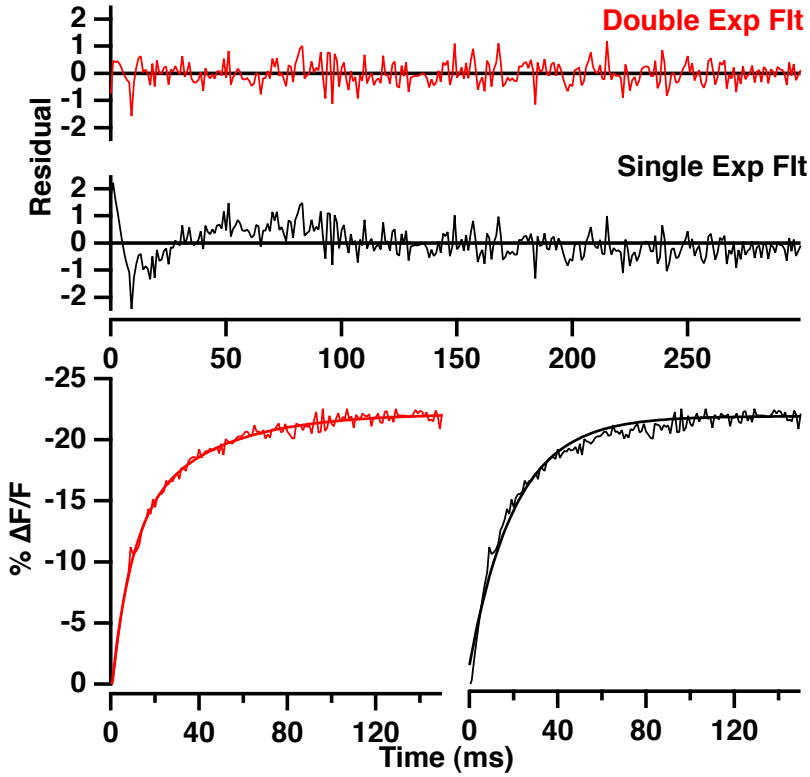
Note: “GDP” is translated from a BamH I restriction site.

Supplemental figure 1



Supplemental figure 2

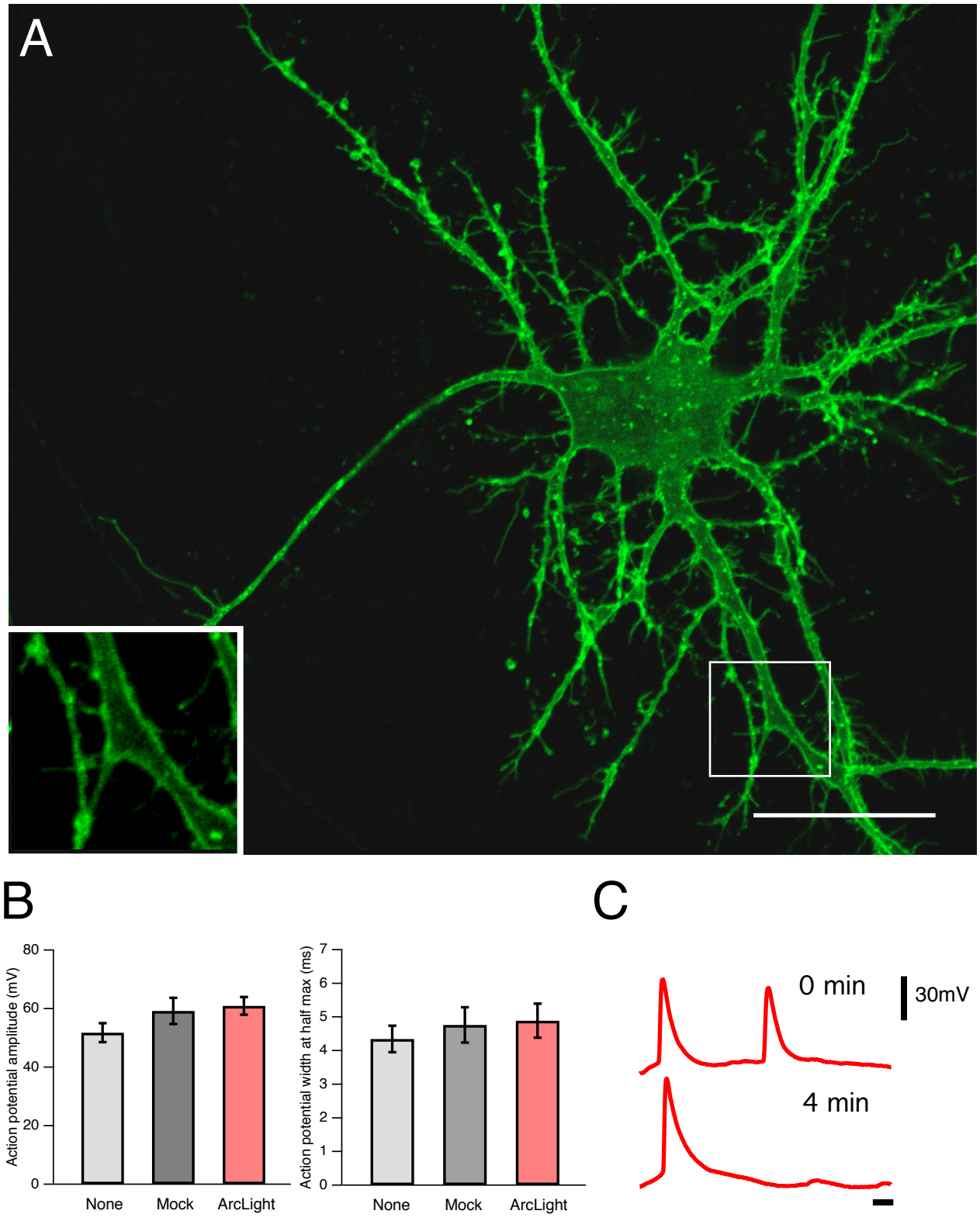
A



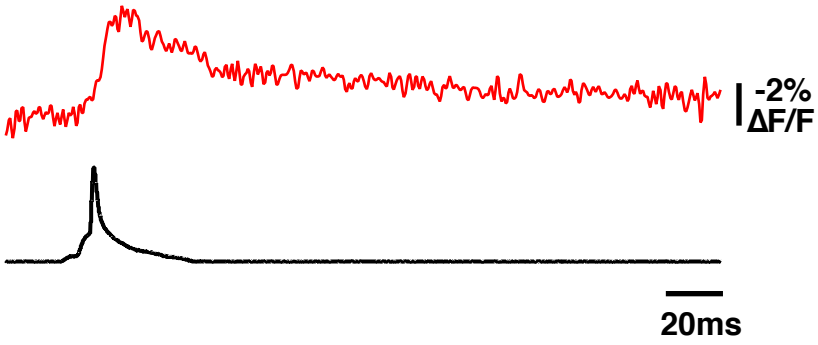
B

	On 1		On 2	Off 1		Off 2
	τ	Amplitude	τ	τ	Amplitude	τ
Ecliptic (A227D) S249	14 ± 2	0.68 ± 0.06	78 ± 28	27 ± 3	0.55 ± 0.04	72 ± 19
ArcLight S249	10 ± 1	0.63 ± 0.05	47 ± 4	19 ± 2	0.53 ± 0.09	62 ± 9
ArcLight Q239	9 ± 1	0.50 ± 0.03	48 ± 4	17 ± 1	0.79 ± 0.03	60 ± 7
ArcLight M240	10 ± 1	0.55 ± 0.03	52 ± 4	20 ± 1	0.68 ± 0.05	77 ± 11
ArcLight K241	12 ± 1	0.51 ± 0.04	51 ± 6	20 ± 1	0.83 ± 0.03	68 ± 10
ArcLight A242	10 ± 1	0.65 ± 0.03	53 ± 4	22 ± 1	0.70 ± 0.04	74 ± 4
ArcLight S243	9 ± 1	0.62 ± 0.03	44 ± 5	18 ± 1	0.68 ± 0.04	53 ± 9

Supplemental figure 3



Supplemental figure 4



Supplemental figure 5